

Human Interleukin-3 (hIL-3)

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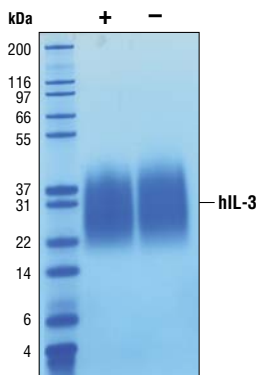
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Source: Recombinant human interleukin-3 (hIL-3) Ala20-Phe152 (Accession # NP_000579) was expressed in human 293 cells at Cell Signaling Technology.

Molecular Characterization: Recombinant hIL-3 contains no "tags" and the nonglycosylated protein has a calculated MW of 15,091. DTT-reduced and non-reduced protein migrate as 26 kDa polypeptides. Lower mobility and heterogeneity in SDS PAGE are due to glycosylation. The expected amino-terminal APMTQ of recombinant hIL-3 was verified by amino acid sequencing.

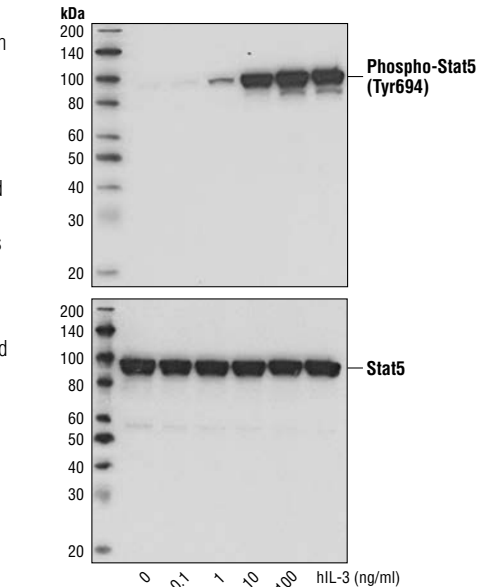
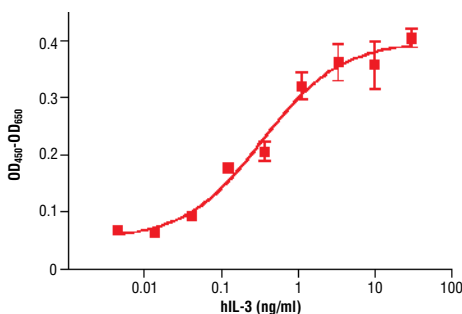
Endotoxin: Less than 0.01 ng endotoxin/1 µg hIL-3.

Purity: >98% as determined by SDS-PAGE of 6 µg reduced (+) and non-reduced (-) recombinant hIL-3. All lots are greater than 98% pure.



The purity of recombinant hIL-3 was determined by SDS-PAGE of 6 µg reduced (+) and non-reduced (-) recombinant hIL-3 and staining overnight with Coomassie Blue.

Bioactivity: The bioactivity of recombinant hIL-3 was determined in a TF-1 cell proliferation assay. The ED₅₀ of each lot is between 50-400 pg/ml.



Western blot analysis of extracts from TF-1 cells untreated or treated with hIL-3 for 15 minutes, using Phospho-Stat5 (Tyr694) (C11C5) Rabbit mAb #9359 (upper) and Stat5 (3H7) Rabbit mAb #9358 (lower).

Formulation: With carrier: Lyophilized from a 0.22 µm filtered solution of PBS, pH 7.2 containing 20 µg BSA per 1 µg hIL-3.

Carrier free: Lyophilized from a 0.22 µm filtered solution of PBS, pH 7.2.

Reconstitution:

With carrier: Add sterile PBS, or PBS containing 1% bovine or human serum albumin or 5-10% FBS to a final hIL-3 concentration of greater than 50 µg/ml. Solubilize for 30 minutes at room temperature with occasional gentle vortexing.

Carrier free: Add sterile PBS, or PBS containing protein to minimize absorption of hIL-3 to surfaces. Solubilize for 30 minutes at room temperature with occasional gentle vortexing. Stock hIL-3 should be greater than 50 µg/ml.

Storage: Stable in lyophilized state at -20°C for 1 year after receipt. Sterile stock solutions reconstituted with carrier protein are stable at 4°C for 2 months and at -20°C for 6 months. Avoid repeated freeze-thaw cycles.

Maintain sterility. Storage at -20°C should be in a manual defrost freezer.

Applications: Optimal concentration for the desired application should be determined by the user.

Background: IL-3 is produced by T cells, mast cells and eosinophils (1). Target cells include hematopoietic progenitors, neutrophils, macrophages, mast cells, eosinophils, lymphoid and erythroid cells (1). The IL-3 receptor is a heterodimer of the IL-3 specific α-chain and the common β-chain, βc, which is also used by GM-CSF and IL-5. (1). Binding of IL-3 can also involve substitution of the βc by a βL-3-chain that appears to be specific for IL-3 (1,2). Binding of IL-3 to its cognate receptor(s) induces activation of Jak2, phosphorylation of multiple Stats (1,3,5,6), and the PI3K/Akt pathway (1). IL-3 may play an important role in the development of airway inflammation associated with asthma (3,4,5).

Background References:

- (1) Reddy, E.P. et al. (2000) *Oncogene* 19, 2532-47.
- (2) Hara, T. and Miyajima, A. (1992) *EMBO J* 11, 1875-84.
- (3) Asquith, K.L. et al. (2008) *J Immunol* 180, 1199-206.
- (4) Schroeder, J.T. et al. (2009) *J Immunol* 182, 2432-8.
- (5) Munitz, A. et al. (2006) *J Immunol* 177, 77-83.

◀ The proliferation of TF-1 cells treated with increasing concentrations of hIL-3 was assessed. After 48 hour treatment with hIL-3, cells were incubated with a tetrazolium salt and the OD₄₅₀-OD₆₅₀ was determined.